

CLEAN VERSION OF CLAIMS

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1. A method for creating a target single-stranded region in a double-stranded DNA, comprising:

A1 (a) nicking at least one site bordering the target region within the double-stranded DNA with at least one site-specific nicking endonuclease; and

(c) subjecting the nicked DNA to conditions where the target region is selectively denatured; to create the target single-stranded region in the double-stranded region.

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A2 22. A nucleic acid molecule having at least one recognition site and an associated nicking site for a site specific single strand nicking endonuclease, the nucleic acid further comprising a target single strand region in a double-stranded DNA formed according to claim 1.

B1 23. A nucleic acid molecule according to claim 22, having a first and a second single strand nicking site wherein the first nicking site is orientated in tandem with respect to the second nicking site.

24. A nucleic acid molecule according to claim 22, having a first and optionally a second single strand nicking site such that the number of nucleotides between the first and the second nicking site or the first nicking site and a terminus of the nucleic acid molecule

determines the length of a cohesive region in the target single stranded region created according to claim 1.

A<sub>2</sub>  
25. A nucleic acid molecule according to claim 22 having a first single stranded target region, the first region forming a stable pairing with a second single stranded target region of DNA having a complementary sequence.

Cont.  
B<sub>1</sub>  
26. A nucleic acid molecule according to claim 24, wherein the cohesive region has a length in the range of 12 to 18 nucleotides.

27. A nucleic acid molecule according to claim 26, wherein the cohesive region has a sequence selected from any of SEQ ID NO:16 through SEQ ID NO:32.

28. A nucleic acid molecule according to claim 26, wherein the cohesive region has a sequence selected from any of SEQ ID NO:35 through SEQ ID NO:49.

29. A nucleic acid molecule according to claim 22, wherein the recognition site selectively binds the site specific single strand nicking endonuclease, N.BstNBI.